

# REVIEW & INTERPRETATION

## A Decade of QTL Mapping for Cyst Nematode Resistance in Soybean

Vergel C. Concibido,\* Brian W. Diers, and Prakash R. Arelli

### ABSTRACT

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe), the most destructive pest of soybean [*Glycine max* (L.) Merrill], is estimated to be responsible for almost nine million megagrams in annual yield loss worldwide. Host plant resistance is the most cost-effective and environmentally friendly method of controlling SCN. Resistance is present among soybean plant introductions (PIs) and related wild species, such as *Glycine soja* Sieb. and Zucc. Molecular marker technology has ushered in a decade devoted to the identification and characterization of quantitative trait loci (QTL) underlying SCN. These genetic mapping efforts uncovered numerous locations of SCN resistance QTL in many PIs. In more than a decade of mapping SCN resistance QTL, there is some consistency in the results. In almost all studies involving various sources of resistance, the QTL conferring the greatest level of resistance mapped to the region containing *rhg1* on linkage group (LG) G. In addition, a major resistance QTL was mapped in many sources to the region containing *Rhg4* on LG A2. The mapping of QTL to these regions from many sources suggests that these sources may have resistance genes in common, which has caused concern over the possible dependence on a few resistance genes. Recently, two independent research groups reported cloning candidate genes for *rhg1* and *Rhg4*. Despite these advances, there is some degree of trepidation, especially in the public sector, on the use of *rhg1* and *Rhg4* genetic mapping and cloning information in SCN resistance breeding because of intellectual property issues.

SOYBEAN CYST NEMATODE is estimated to cause the greatest yield loss to soybean compared to any other pest worldwide. Most recent estimates include a loss of 7.6 million megagrams in the USA and nearly nine million worldwide in 1998 (Wrather et al., 2001). Soybean cyst nematode causes yield reductions by feeding on plant nutrients, retarding root growth, and inhibiting *Bradyrhizobium japonicum* (Kirchner) Buchanan nodulation (Riggs and Schmidt, 1987).

The genetically diverse field populations of *H. glycines* combined with the limited germplasm base of commercial soybean for resistance could potentially lead to population shifts over time (Colgrove et al., 2002). Diversity in SCN has been described with a physiological race system that utilizes four resistant soybean genotypes and a susceptible standard as differential lines (Golden et al., 1970). However, this race system does not account for all of the diversity present in SCN. For

example, Rao-Arelli et al. (1992b) showed that several isolates of *H. glycines* were initially classified as one race by this scheme. However, when later tested with resistant genotypes other than the standard differentials, the isolates behaved as different races. To describe diversity better within the nematode, the race system has been replaced with a new scheme that includes additional differentials or indicator lines and classifies nematode populations as "*H. glycines* types" (HG types) (Nikblack et al., 2002). In this review, SCN populations used in mapping studies will be referred to by race designations because most of this work was done before the development of the new HG type system.

The primary methods for controlling SCN include planting resistant cultivars and rotation with nonhost crops. Breeders and nematologists have been successful in developing SCN resistant cultivars. Host plant resistance has allowed soybean production to continue in many growing areas where production could no longer be profitable because of SCN infestations. It was estimated that the resistant cultivar Forrest alone prevented crop losses worth \$405 million from 1975 to 1980 (Bradley and Duffy, 1982).

Plant introductions from the USDA Soybean Germplasm Collection have been screened for resistance to nematode populations and resistant lines have been identified (Ross and Brim, 1957; Epps and Hartwig, 1972; Anand and Gallo, 1984; Anand et al., 1988; Young, 1990; Rao-Arelli et al., 1997). Arelli et al. (2000) identified 118 exotic soybean PIs resistant to combinations of SCN races 1, 2, 3, 5, or 14. Anand et al. (1988) reported that the only PI they found with resistance to nearly all SCN races tested was PI 437654, a claim that was later validated by the work of Diers et al. (1997b). Diers et al. (1997b) evaluated a set of 38 SCN resistant PIs with *H. glycines* races 1, 2, 3, 5, and 14 in the greenhouse. Among these 38 PIs, only PI 437654 and PI 438489B were classified as resistant to all five SCN races in their test. PI 437654 was the more resistant of the two, with a higher number of cysts produced on PI 438489B. In addition, previous work showed that PI 438489B was susceptible to other isolates of races 2 and 14 (Rao-Arelli et al., 1992b). Diers et al. (1997b) also evaluated the genetic relationships among the PIs with 201 genomic clones used as restriction fragment length polymorphism (RFLP) markers. They found that the clustering of the PIs based on markers was consistent with resis-

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**Abbreviations:** AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; LG, linkage group; LRR, leucine-rich repeat; MAS, marker-assisted selection; QTL, quantitative trait locus; R-gene, resistance gene; SCN, soybean cyst nematode; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

tance responses to *H. glycines*. A cluster of PIs that most likely share resistance genes with 'Peking' and PI 88788 was also identified.

To date, breeders have incorporated resistance genes from only a few PIs into commercial soybean cultivars. The genetic vulnerability of current soybean germplasm to SCN was illustrated by Anand (1991), who conducted a survey among soybean breeders in North America to determine the SCN resistance sources employed in their breeding programs. The survey showed that of 130 SCN resistant cultivars developed, 57 came from the public sector and 73 from the private sector. The resistance in 69 of these cultivars can be traced to Peking, 24 to PI 88788, 31 to both Peking and PI 88788, and two to PI 90763. In the Midwest, where the majority of the U.S. soybean production is located, PI 88788 is the predominant source of SCN resistance (Diers and Arelli, 1999). In 2001, among the 760 maturity group (MG) I to IV cultivars listed as carrying SCN resistance, 705 had their resistance from PI 88788 alone (Marion Shier, personal communication). More emphasis is now being directed to breeding with additional sources of resistance, such as PI 437654 and PI 89772 (Anand, 1992; Nickell et al., 1999) and PI 209332 (Orf and MacDonald, 1995).

Caldwell et al. (1960) were the first to report on the inheritance of resistance to *H. glycines*. They found that the inheritance of resistance from Peking fits a three recessive gene model and symbols *rhg1*, *rhg2*, and *rhg3* were assigned. A later report indicated the presence of a fourth resistance gene from Peking that is closely linked to the *i* locus (Matson and Williams, 1965), a locus which controls pigment distribution on the seed coat. This fourth gene was reported as a dominant resistance gene and was designated as *Rhg4*. An additional dominant gene in PI 88788 was identified (Rao-Areli et al., 1992a) and was later designated as *Rhg5* (Rao-Areli, 1994). Dong et al. (1997) summarized the work that has been done on the genetics of resistance in a number of PIs. These efforts show that some resistance genes are in common among PIs and others were unique (Rao-Areli and Anand, 1988; Anand and Rao-Areli, 1989; Rao-Areli et al., 1989; Myers and Anand, 1991; Rao-Areli et al., 1992b; Young and Kilen, 1994). The difficulties involved in conducting allelism tests with the established SCN resistance genes make it difficult to prove the novelty of newly discovered genes from PIs in more recent studies.

Genetic marker technology has facilitated the identification, localization, and characterization of QTL associated with important agronomic traits, such as SCN resistance. The first comprehensive genetic maps in soybean were constructed by means of RFLP markers (Keim et al., 1990; Shoemaker and Olson, 1993). Additional mapping has been done with random amplified polymorphic DNA (RAPD) markers (Ferreira et al., 2000), amplified fragment length polymorphism (AFLP) markers (Keim et al., 1997), and simple sequence repeat (SSR) markers (Cregan et al., 1999a). Currently, SSR markers are the most widely used marker system in soybean because they are easy to use, highly polymorphic, and they map to a single locus with each primer pair.

This ability to map to a single locus has been especially beneficial because it has made it relatively easy to compare mapping results across genetic populations. The wide availability of genetic marker resources in soybean has led to the publication of numerous papers on the identification and localization of QTL underlying resistance to SCN. This review summarizes these SCN QTL discoveries and discusses how we can better utilize this wealth of genetic mapping information in breeding for SCN resistance. This review also focuses on how these publications have ushered in the new era of breeding and selection for SCN resistant soybean genotypes, also known as marker-assisted selection (MAS), and the eventual sequencing and cloning of candidate *rhg1* and *Rhg4* resistance alleles.

### SCN QTL Discovery

We have summarized in Table 1 all SCN resistance QTL that we could find in the published literature from 1992 up to the time of preparation of this review. There were a total of 16 SCN resistance QTL mapping papers published (Table 1) with 60 nonindependent reports of marker-SCN resistance associations on the following LGs: A1, A2, B1, B2, C1, C2, D1a, D2, E, F, G, H, I, J, L, M, and N (Tables 1–2, Fig. 1–2). Linkage group G has the most QTL regions associated with SCN resistance with four, LGs B1, C2, and D2 with three, and LGs A1, B2, D1a, E, and M have two while the rest of the LGs have one (Table 2, Fig. 1). The approximate map locations of the reported putative QTL are shown on Fig. 1. Because of differences in size and structure of mapping populations, DNA marker platforms, and limited availability of DNA markers at the time of publication of many of the reports included in this review, the placement of these QTL on the soybean genetic linkage map is tentative at best. Among the resistance sources studied, Peking had the most resistance QTL mapped with nine QTL mapping to independent regions, followed by eight for PI 438489B, and six for PI 437654 (Table 2). Five or fewer QTL were discovered in the remaining PIs. Peking was also the most studied, with five research groups that conducted genetic mapping with this PI in addition to others who studied resistance from Peking in combination with other sources (Table 2). The reported QTL effects ranged from 1 to 91% of the total phenotypic variation for resistance in the population used (Table 1, Fig. 2).

The first publication on the use of molecular markers to map SCN resistance genes was a report of a tight linkage between the two molecular markers, pBLT24 and pBLT65, and the SCN resistance gene, *Rhg4* (Weismann et al., 1992). However, a direct assay was not performed for nematode resistance and the association was based solely on the linkage of the markers to the *i* locus, which previously had been reported to be linked with *Rhg4* (Matson and Williams, 1965).

Concibido et al. (1994) were the first to identify and localize QTL for SCN resistance based on both genotypic and phenotypic data in a segregating population. In a population segregating for resistance from

Table 1. List of all soybean cyst nematode (SCN) marker associations reported in literature (1992–present).

LG†	Predicted gene	Race significant‡	Percent effect	LOD	P-value	Peak marker/interval	Marker type	Cross§	Source	Authors	Reference
A1	unknown	3	1.0	not available	0.0008	A487	RFLP	Williams 82 × (Hartwig)	Peking, PI 437654	Vierling et al., 1996	Theor. Appl. Genet. 92:83–86
A1	unknown	2	7.4	2.8	not available	A262-Satt300	RFLP/SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
A2	<i>Rhg4</i>	not available	not available	not available	not available	pBLT24, pBLT65	RFLP	PI 290136 × BARC-2 (Rj4)	not available	Weismann et al., 1992	Theor. Appl. Genet. 85:136–138
A2	<i>Rhg4</i>	3	24.0	not available	0.0001	pBLT65a	RFLP	Essex × (Forrest)	Peking	Chang et al., 1997	Crop Sci. 37:965–971
A2	<i>Rhg4</i>	3	25.0	not available	0.0001	<i>Ilocus</i>	Morphological	(Peking) × Essex	Peking	Mahalingam and Skorupska, 1995	Breed. Sci. 45:435–443
A2	<i>Rhg4</i>	3	16.0	not available	0.0001	EcggMaac405	AFLP	(Forrest) × Essex	Peking	Meksem et al., 2001	Theor. Appl. Genet. 103:710–717
A2	<i>Rhg4</i>	1,3	17.5–18.9	not available	0.0001	SCAR548/563	SCAR	(J87-233) × Hutcheson	Peking, PI 88788, PI 90763	Heer et al., 1998	Mol. Breed. 4:359–367
A2	<i>Rhg4</i>	3	15.0	2.9	0.002	A085	RFLP	M83-15 × (M85-1430)	PI 209332	Concibido et al., 1994	Crop Sci. 34:240–246
A2	<i>Rhg4</i>	3	9.0	not available	<0.05	<i>Ilocus</i>	Morphological	BSR101 × (PI 437654)	PI 437654	Webb et al., 1995	Theor. Appl. Genet. 91:574–581
A2	<i>Rhg4</i>	1,3	16.0–28.0	not available	0.0001	pBLT65a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
unknown	unknown	3	91.0	not available	0.0001	A006	RFLP	Williams 82 × (Hartwig)	Peking, PI 437654	Vierling et al., 1996	Theor. Appl. Genet. 92:83–86
B1	unknown	1,2,5	6.8–16.6	2.8–6.8	not available	A006-Satt583	RFLP/SSR	Hamilton × (PI 89772)	PI 89772	Yue et al., 2001b	Crop Sci. 41:1589–1595
B1	unknown	1,2,5	7.4–12.7	2.7–4.2	not available	Satt583-Sat_123	SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
B1 (L25)	unknown	1,2,5,14	2.0–7.0	not available	0.0001	A567a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
B1 (S)	unknown	3	1.0	not available	0.0001	A567	RFLP	Williams 82 × (Hartwig)	Peking, PI 437654	Vierling et al., 1996	Theor. Appl. Genet. 92:83–86
B2	unknown	1,3	9.2–21.0	not available	0.009–0.0001	A593	RFLP	(Peking) × Essex	Peking	Qui et al., 1999	Theor. Appl. Genet. 98:356–364
B2	unknown	1,3	8.1–11.7	2.6–2.8	not available	Satt168-A329	RFLP/SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
C1	unknown	2,14	10.2–11.1	2.6–3.6	not available	A463-Satt396	RFLP/SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
C1	unknown	1,2	1.0–2.0	not available	0.03	php02298b	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
C2	unknown	3	5.0	4.4	not available	A121_1	RFLP	PI 468916 × (A81-356022)	A81-356022	Wang et al., 2001	Theor. Appl. Genet. 103:561–566
C2	unknown	3	8.0	not available	0.009	A635	RFLP	(Peking) × Essex	Peking	Mahalingam and Skorupska, 1995	Breed. Sci. 45:435–443
C2	unknown	14	6.5	not available	0.006	A426	RFLP	(J87-233) × Hutcheson	Peking, PI 88788, PI 90763	Heer et al., 1998	Mol. Breed. 4:359–367
C2	unknown	1,5	7.1–8.3	3.0–6.8	not available	Satt371-Satt202	SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
D1a	unknown	3,5,14	7.4–10.7	4.1–5.5	not available	A398-K487	RFLP	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
D1a	unknown	5	7.8	3.3	not available	Satt342-Satt368	SSR	Hamilton × (PI 89772)	PI 89772	Yue et al., 2001b	Crop Sci. 41:1589–1595
D2	unknown	14	41.1	not available	<0.0001	Satt082	SSR	(Hartwig) × BR 92-31983	Peking, PI 437654	Schuster et al., 2001	Theor. Appl. Genet. 102:91–96
D2	unknown	6	11.0	not available	0.001	A064-2	RFLP	Evans × (PI 209332)	PI 209332	Concibido et al., 1996b	Crop Sci. 36:1643–1650
D2	unknown	1	9.7	4.6	not available	B132-Satt372	RFLP/SSR	Hamilton × (PI 89772)	PI 89772	Yue et al., 2001b	Crop Sci. 41:1589–1595
E	unknown	2,14	8.0–18.7	2.6–5.0	not available	A656-Satt452	RFLP/SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
E	unknown	3	23.0	3.1	not available	Bng107_1-A458_1	RFLP	Hamilton × (PI 438489B)	PI 468916	Wang et al., 2001	Theor. Appl. Genet. 103:561–566
E	unknown	3	15.7	3.6	not available	A135-Satt231	RFLP/SSR	Hamilton × (PI 89772)	PI 89772	Yue et al., 2001b	Crop Sci. 41:1589–1595
F	unknown	3	6.0	not available	0.03	G15	RAPD	(Peking) × Essex	Peking	Mahalingam and Skorupska, 1995	Breed. Sci. 45:435–443
F	unknown	3	1.0	not available	0.002	A112	RFLP	Williams 82 × (Hartwig)	Peking, PI 437654	Vierling et al., 1996	Theor. Appl. Genet. 92:83–86
F	unknown	1,5	6.5–7.2	not available	0.02	K002	RFLP	(J87-233) × Hutcheson	Peking, PI 88788, PI 90763	Heer et al., 1998	Mol. Breed. 4:359–367
G	<i>Rhg1</i>	3	16.0	not available	0.003	K069	RFLP	M83-15 × (M85-1430)	PI 209332	Concibido et al., 1994	Crop Sci. 34:240–246
G	<i>Rhg1</i>	3	14.0	not available	0.0001	O10.3450	RAPD	Essex × (Forrest)	Peking	Chang et al., 1997	Crop Sci. 37:965–971
G	<i>Rhg1</i>	1,3,6	17.8–26.2	not available	<0.0001	C006	RFLP	Evans × (Peking)	Peking	Concibido et al., 1997	Crop Sci. 37:258–264
G	unknown	1,3,6	10.3–17.6	not available	0.001–0.0001	A378	RFLP	Evans × (Peking)	Peking	Concibido et al., 1997	Crop Sci. 37:258–264
G	<i>Rhg1</i>	3	26.2	not available	0.0001	EcggMcga87	AFLP	(Forrest) × Essex	Peking	Meksem et al., 2001	Theor. Appl. Genet. 103:710–717
G	<i>Rhg1</i>	3	28.0	not available	0.0001	Satt038	SSR	Flyer × (Hartwig)	Peking, PI 437654	Prabhu et al., 1999	Crop Sci. 39:982–987
G	<i>Rhg1</i>	1,3	5.3–11.9	not available	0.07–0.002	K069	RFLP	(J87-233) × Hutcheson	Peking, PI 88788, PI 90763	Heer et al., 1998	Mol. Breed. 4:359–367
G	<i>Rhg1</i>	1,3,6	35.0–54.0	not available	0.0001	C006	RFLP	Evans × (PI 209332)	PI 209332	Concibido et al., 1996b	Crop Sci. 36:1643–1650
G	<i>Rhg1</i>	3	22.0	not available	<0.05	php05354a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb et al., 1995	Theor. Appl. Genet. 91:574–581
G	<i>Rhg1</i>	1,2,3,5,14	10.0–43.0	not available	0.0001	php05354a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
G	unknown	1,2,5	5.8–15.8	2.3–9.1	not available	Satt130-Satt012	SSR	Hamilton × (PI 438489B)	PI 438489B	Yue et al., 2001a	Theor. Appl. Genet. 102:921–928
G	unknown	3	27.0	not available	not available	Satt288-Satt472	RFLP	Hamilton × (PI 468916)	PI 468916	Wang et al., 2001	Theor. Appl. Genet. 103:561–566
G	<i>Rhg1</i>	3,6	26.2–36.3	not available	<0.0001	C006	RFLP	Evans × (PI 88788)	PI 88788	Concibido et al., 1997	Crop Sci. 37:258–264
G	<i>Rhg1</i>	1,2,5,5	4.6–26.6	2.5–13.7	not available	B053-Satt309	RFLP/SSR	Hamilton × (PI 89772)	PI 89772	Yue et al., 2001b	Crop Sci. 41:1589–1595
G	<i>Rhg1</i>	1,3,6	13.6–44.8	15.8	<0.0001	C006	RFLP	Evans × (PI 90763)	PI 90763	Concibido et al., 1997	Crop Sci. 37:258–264

Continued next page.

Table 1. Continued.

LG†	Predicted gene	Race significant‡	Percent effect	LOD	P-value	Peak marker/interval	Marker type	Cross§	Source	Authors	Reference
H	unknown	1,3	9.0–13.0	not available	0.003–0.002	B072	RFLP	(Peking) × Essex	Peking	Qui et al., 1999	Theor. Appl. Genet. 98:356–364
I	unknown	5	11.0	not available	0.001	K011	RFLP	(Peking) × Essex	Peking	Qui et al., 1999	Theor. Appl. Genet. 98:356–364
J	unknown	3	38	6.1	<0.0001	B032	RFLP	M83-15 × (M85-1430)	PI 209332	Concibido et al., 1994	Crop Sci. 34:240–246
J	unknown	3	35.0	not available	<0.0001	B032	RFLP	Evans × (PI 209332)	PI 209332	Concibido et al., 1996b	Crop Sci. 36:1643–1650
J	unknown	3	18.8	not available	<0.0001	B032	RFLP	Evans × (PI 90763)	PI 90763	Concibido et al., 1997	Crop Sci. 37:258–264
J (L26)	unknown	2,14	3.0–9.0	not available	0.0001	K079	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
L	unknown	1	24.0	not available	0.002	A023	RFLP	Evans × (PI 209332)	PI 209332	Concibido et al., 1996b	Crop Sci. 36:1643–1650
M	unknown	1,3,5	5.4–6.9	not available	0.02	A131	RFLP	(J87-233) × Hutcheson	Peking, PI 88788, PI 90763	Heer et al., 1998	Mol. Breed. 4:359–367
M	unknown	3	7.0	not available	<0.05	php020301a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb et al., 1995	Theor. Appl. Genet. 91:574–581
M	unknown	1,3,5,14	0.0–14.0	not available	0.0001	php020301a	RFLP	BSR101 × (PI 437654)	PI 437654	Webb, 2003	U.S. Patent 6,538, 175 B1
N	unknown	6	14.3	not available	<0.0001	A280	RFLP	Evans × (Peking)	Peking	Concibido et al., 1997	Crop Sci. 37:258–264

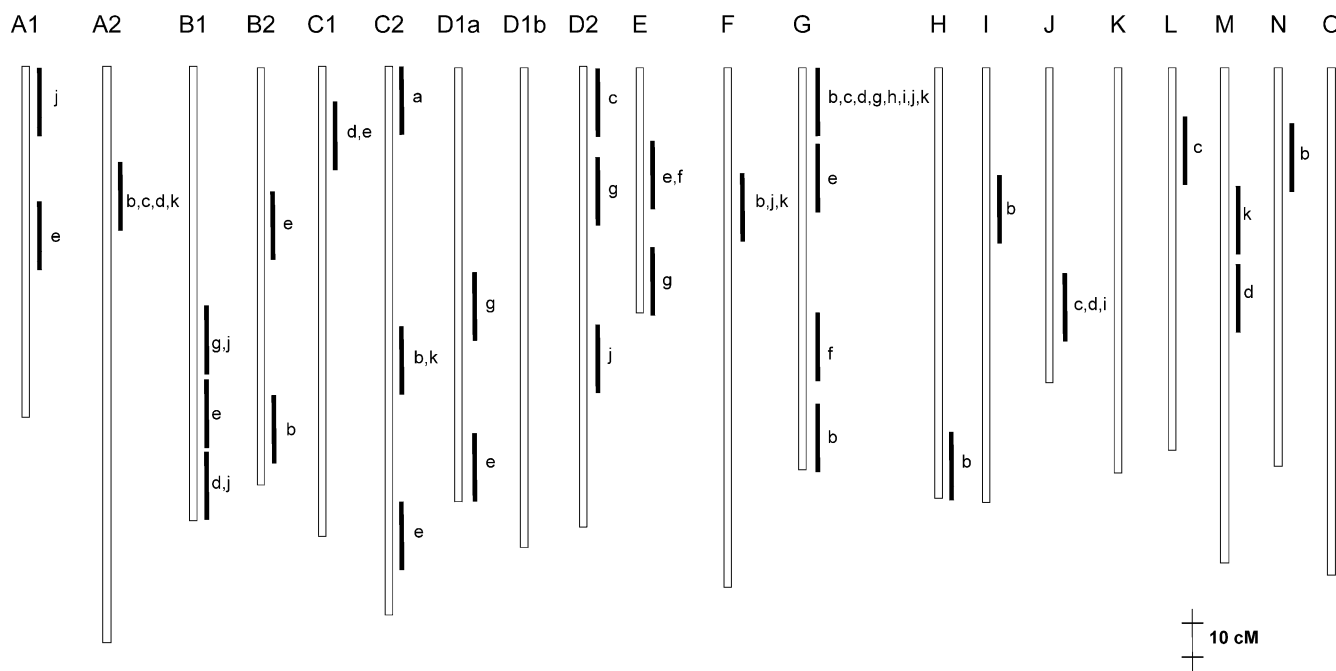
† Linkage group designations are based on most recent placement on the composite soybean molecular linkage map (Cregan et al., 1999a). If the original linkage assignment is different, then this is enclosed in parentheses.  
‡ The SCN race which has the most significant association with the QTL region is underscored when more than one race was significant for a QTL region.  
§ The SCN resistant parent in each cross is in parentheses.

Table 2. Number of soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) quantitative trait loci (QTL) discoveries across sources of resistance.

Source of SCN resistance	Linkage group†										QTL/ source	Labs/ source
A81-356022											1	1
Peking											9	5
PI 209332											5	1
PI 437654											6	1
PI 438489B											8	1
PI 468916											2	1
PI 88788											1	1
PI 89772											5	1
PI 90763											2	1
Peking + PI 437654											6	3
Peking + PI 88788 + PI 90763											5	1

† Linkage groups (LGs) that are followed by the same superscript indicate that they have QTL regions that are mapping to the same location.  
‡ Numbers in parentheses indicate the total number of citations attributed to the QTL region per resistance source.





**Legend:**

A81-356022 - (a) Peking - (b) PI 209332 - (c) PI 437654 - (d) PI 438489B - (e) PI 468916 - (f)

PI 89772 - (g) PI 88788 - (h) PI 90763 - (i) Peking+PI 437654 - (j) Peking+PI 88788+PI 90763 - (k)

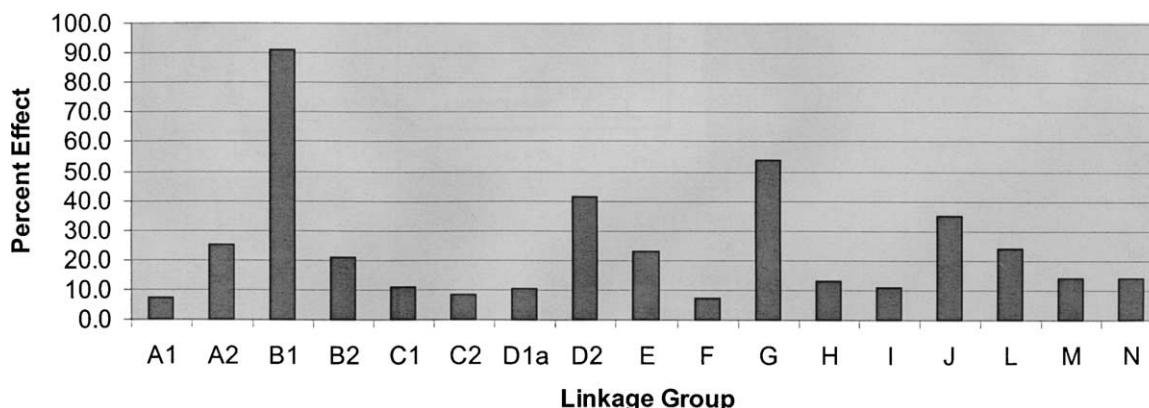
**Fig. 1.** The soybean genetic linkage map (Cregan et al., 1999a) showing approximate locations for all published soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) quantitative trait loci (QTL). Thick white lines represent linkage groups, short dark lines represent 20-cM intervals where the SCN QTL are predicted to be located. Due to differences in size and structure of mapping populations, DNA marker platforms, and limited availability of DNA markers at the time of publication of many of the reports included in this review, the placement of these QTL on the soybean genetic linkage map is tentative.

PI 209332, the RFLP markers A085 and B032 were found to be significantly associated with the resistance response to a SCN population with a race 3 phenotype from Minnesota. The two markers together accounted for 51.7% of total phenotypic variation for resistance in the population. The marker A085, located on LG A2 of the soybean RFLP map (Shoemaker and Olson, 1993), was linked to *i* locus at a distance of 10.9 cM. This finding confirmed the earlier study of Weismann et al.

(1992). Later studies with Peking (Mahalingam and Skorupska, 1995; Chang et al., 1997), PI 437654 (Webb et al., 1995), and 'J87-233', soybean line with Peking, PI 88788 and PI 90763 in its pedigree (Heer et al., 1998), confirmed the presence of a QTL conferring race 3 resistance near the *i* locus on LG A2 (Cregan et al., 1999a).

The second marker, B032, tentatively located on LG K of the soybean RFLP map (Shoemaker and Olson,

**Highest Reported SCN QTL Effect Per Linkage Group**



**Fig. 2.** Quantitative trait loci (QTL) for soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) resistance reported in the literature as showing the strongest phenotypic effects for each linkage group of the soybean genetic linkage map (Cregan et al., 1999a).

1993), showed a strong association with SCN disease response in the PI 209332 population and accounted for 38% of the total phenotypic variation for resistance (Concibido et al., 1994). After additional marker data became available, Concibido (1995) revised the position of B032 to its correct position on LG J. In a later study, Concibido et al. (1997) reported that a resistance QTL from PI 90763 also mapped near B032 on LG J.

Concibido et al. (1994) reported a third genomic region, defined by the RFLP marker K069, associated with SCN resistance. The RFLP K069 was previously reported to be associated with SCN resistance in a study using near-isogenic soybean lines (Boutin et al., 1992). They placed the marker on LG G of the soybean RFLP map (Shoemaker and Olson, 1993). This led to the discovery of other markers tightly linked with K069, uncovering a major resistance QTL on LG G (Concibido et al., 1994). Consistent with early genetic studies, the resistant QTL allele on LG G was assigned the gene symbol *rhg1*.

Follow up research showed that a QTL for race 3 resistance mapped to the location of *rhg1* in PI 209332, Peking, PI 88788, and PI 90763 (Concibido et al., 1995; 1997). Additional studies showed that PI 437654 also carried race 3 resistance in the same region on LG G (Webb et al., 1995) and confirmed the presence of a resistance QTL in this region in PI 88788, Peking, and J87-233 (Diers et al., 1997a; Chang et al., 1997; Heer et al., 1998).

Concibido et al. (1996a) later initiated high density mapping around *rhg1* to characterize further the region, placing the SCN resistance locus 4.6 cM from the RFLP marker B053 and 2.8 cM from RFLP marker Bng30. More recently, SSR markers have been mapped close to *rhg1* (Mudge et al., 1997; Cregan et al., 1999b). This includes the SSR marker Satt309, which is estimated to map 0.4 cM from *rhg1* (Cregan et al., 1999b).

### QTL Mapping Inconsistencies

Despite the relatively consistent mapping results observed with *rhg1* and *Rhg4*, there are inconsistencies reported in several studies. For example, Webb et al. (1995) reported three QTL for SCN resistance against race 3 on LGs A2, G, and M in PI 437654. They found that the locus on LG G (*rhg1*) had a greater effect than the locus on LG A2 (*Rhg4*), but neither locus alone provided a degree of resistance that could account for most of the phenotypic variation. However, Vierling et al. (1996a) found different regions associated with SCN race 3 resistance in PI 437654 than those reported by Webb et al. (1995). Using a population derived from a cross between 'Williams 82' and 'Hartwig', [Hartwig was derived from backcrossing PI 437654 resistance into Forrest, which has SCN resistance from Peking (Anand, 1992)], they reported resistance QTL on LGs A2, B1, F, and S. The RFLP marker on LG S (A567) that Vierling et al. (1996a) reported was later placed on LG B1 of the soybean genetic linkage map (Cregan et al., 1999a). As mentioned earlier, Forrest derives its SCN resistance from Peking, and like PI 437654, has SCN resistance QTL corresponding to *rhg1* and *Rhg4* (Webb

et al., 1995). The QTL on LG B1 found by Vierling et al. (1996a) is quite interesting because it explained more than 90% of the total phenotypic variation for resistance in the population, which is the highest effect ever reported for a single SCN resistance QTL (Table 1). This large effect is atypical for quantitative traits, which are usually controlled by many genes, with each gene having a small effect on the trait. This QTL is the main basis for the "Cyst-X" resistance, which was patented by Vierling et al. (1996b). Later, a study by Prabhu et al. (1999) reaffirmed the importance of the combined effect of both the QTL on LGs A2 and G against SCN race 3 in another population with Hartwig as the SCN resistance source.

There are several explanations for the discrepancies in the results of the PI 437654 mapping studies of Webb et al. (1995) and Vierling et al. (1996a). Although both groups tested their mapping populations with race 3 nematode populations, their nematode populations could have different parasitism genes that interacted with different resistance genes in PI 437654 (Niblack et al., 2002). Gene duplication (Shoemaker et al., 1996) could provide a second explanation such that Vierling et al. (1996a) might have mapped a duplicate locus for *rhg1* in PI 437654. In addition, Vierling et al. (1996a) used Hartwig as a source of resistance in their study, which has Peking in its pedigree. It is possible that Peking could have contributed some of the resistance QTL that Vierling et al. (1996a) mapped. For example, the QTL region on LG F was found significantly associated with SCN phenotypic response in three separate studies involving Peking as one of the sources of SCN resistance (Tables 1–2, Fig. 1). A fourth explanation could be that the inconsistencies were the result of epistatic interactions among the SCN resistance genes and the different genetic backgrounds used in the two studies. These interactions could have resulted in different genes having an effect in each study.

Additional resistance genes have been mapped from PI 437654, and these include a QTL on LG D2 mapped by Schuster et al. (2001). They used a bulk segregant analysis to identify a novel QTL for SCN resistance against race 14 on LG D2 in a cross between Hartwig and a susceptible line, 'BR-92-31983'. In addition, Webb (2003) found minor QTL on C1, L25 [an unofficial LG with known RFLP markers from LGs B1, B2, and I of the soybean genetic linkage map (Cregan et al., 1999a)], and L26 [presumably LG J due to linkage to an established RFLP marker on LG J of the soybean genetic linkage map (Cregan et al., 1999a)] in PI 437654. Webb (2003) found that these minor QTL were effective against multiple races of SCN (Table 1). Because PI 437654 is reported to be resistant to all known races of *H. glycines* (Anand et al., 1988), it is not surprising that several resistance QTL have been mapped from this source.

Inconsistencies were also observed for Peking in comparing the QTL mapping results for race 3 resistance across four research groups. Mahalingam and Skorupska (1995) mapped a major resistance QTL from Peking near the *i* locus, presumably *Rhg4*, in addition to QTL

on LGs F and C2. Concibido et al. (1997) found that the locus on G, presumably *rhg1*, is the major QTL conditioning resistance in Peking. Later, Qiu et al. (1999) mapped SCN resistance QTL on LGs B2, H, and I. Qiu et al. (1999) stated that the significant QTL markers in the studies on Peking by Concibido et al. (1997) and Mahalingam and Skorupska (1995) did not reach significant levels in their study. However, a later study by Meksem et al. (2001) involving Forrest, which carries resistance from Peking, reaffirmed the importance of *rhg1* and *Rhg4*. The authors proposed a bigenic model for resistance to race 3. This bigenic model is inconsistent with classical genetic studies (Rao-Arelli et al., 1992a; Caldwell et al., 1960; Matson and Williams, 1965) and other QTL mapping studies. However, since Forrest is derived from Peking, it is possible that Forrest only retained the two resistance QTL from Peking, as reported by Meksem et al. (2001). By having only these two QTL segregate in their population, this may have allowed them to better estimate the effects and interactions of these QTL than was possible in other studies.

There are a number of explanations for the inconsistencies in the mapping of resistance QTL from Peking. First, it is likely that each researcher used a different source of Peking. There exist a number of lines named Peking, but they vary in SCN resistance responses and genetic marker analysis has shown that they are genetically diverse (Skorupska et al., 1994). Another explanation is that these groups (Mahalingam and Skorupska, 1995; Qiu et al., 1999; Concibido et al., 1997), except for Meksem et al. (2001), did not have complete genome coverage with their markers, which may have caused some groups to miss QTL detected by others. Most of the explanations given for the inconsistencies in the PI 437654 mapping work of Webb et al. (1995) and Vierling et al. (1996a) also apply here. These explanations include differences in parasitism genes present in the SCN isolates used in each study, gene duplication, and genetic interactions.

### Novel QTL and Sources of Resistance

With many researchers focused on understanding the resistance conditioned by *rhg1* and *Rhg4*, others pursued novel QTL regions, as well as novel sources of SCN resistance. Yue et al. (2001b) studied the genetic basis of resistance in PI 89772 and found a major QTL mapping to the location of *rhg1*. In addition, they also reported finding QTL on LGs B1, D1a, D2, and E that provide resistance to SCN races 1, 2, and 5. They also concluded that no single locus could provide complete resistance to any particular race, but combinations of these major loci could result in high levels of resistance, an observation shared by Concibido et al. (1996b, 1997) and Webb et al. (1995). Yue et al. (2001a) also found resistance QTL on LGs A1, B1, B2, C1, C2, D1a, E, and G from PI 438489B, another novel source of SCN resistance. PI 438489B is a plant introduction from China with resistance to SCN races 1, 2, 3, 5, and 14 (Diers et al., 1997b). The LG G QTL from PI 438489B does not map to the *rhg1* region, making PI 438489B a

unique resistance source because it is one of the few that is reported not to possess *rhg1*. In an effort to identify novel SCN resistance genes in other PI sources, Diers and Arelli (1999) found that in the SCN resistant accessions PI 92720, PI 22897 ('Columbia'), PI 438503A, and PI 404166, the majority of resistance to SCN races 3 and 14 can be explained by markers tightly linked to resistance genes that breeders are currently using, such as *rhg1* and *Rhg4*.

There have been recent efforts to study whether *G. soja* has novel SCN resistance genes. *Glycine soja* is widely distributed in China, Japan, Korea, Taiwan, and eastern Russia and is believed to be the ancestor of cultivated soybean (Hymowitz and Singh, 1987). Wang et al. (2001) were the first to map SCN resistance QTL from *G. soja*. They mapped SCN resistance QTL in a population developed from a cross between *G. max* (A81-356022) and *G. soja* (PI 468916), which is the same population used in generating the public soybean genetic linkage map (Shoemaker and Olson, 1993; Cregan et al., 1999a). They mapped, in the original population and confirmed in a backcross population, a new major QTL in the region between Satt288 and Satt472 on LG G and a second major QTL on LG E. The QTL on LG G maps to a different region from *rhg1*, and there are no other reports of SCN resistance QTL in this region. The resistance QTL on LG E may not be unique to *G. soja* as Yue et al. (2001a) mapped a QTL from PI 438489B to the same region as the *G. soja* QTL.

### Frequency of QTL Discovery among Sources of Resistance

Although many QTL have been mapped, those that have been mapped to the regions where *rhg1* and *Rhg4* are located were the most consistently cited with 10 and seven independent citations, respectively (Tables 1–2). Quantitative trait loci were mapped to the *rhg1* region from six PI sources, which is the most of any of the resistance QTL (Tables 1–2). In counting the number of sources, we did not include results from mapping studies where multiple sources were used if one or more of the sources were already counted in an independent study. The *Rhg4* region and regions on LGs B1, E, and J each have QTL mapped from at least three sources. Quantitative trait loci were mapped from at least two sources to regions on LG B2, C1, D1a, and D2. The remaining QTL were mapped in a single source and in one population. These QTL that have been mapped in a single source need further confirmation. Additional work is needed to determine whether QTL from different resistance sources that mapped to the same region are allelic. If these sources have genes that are allelic, it is important to know whether different sources have different functional alleles for these genes. If different functional alleles exist, breeders may want to deploy these different alleles in cultivars to increase genetic diversity for resistance.

Resistance QTL have been shown to provide resistance to more than one race of SCN. For example, in the 'Hamilton' × PI 89772 population (Yue et al., 2001b),



the *rhg1* region was significantly associated with resistance to four SCN races. There are many additional examples of broad resistance provided by specific QTL, as well as resistance to only specific races. In the Hamilton × PI 89772 population, the QTL on D1a, D2, and E were each significant for only a single SCN race. Broad resistance to many SCN races would be particularly beneficial in breeding to mitigate the catastrophic consequence of potential race shifts in nematode populations in the field.

### Cloning of Candidate SCN Resistance Genes, *rhg1* and *Rhg4*, and Its Implication to SCN Resistance Breeding

Two independent research groups have reportedly cloned candidate genes for *rhg1* and *Rhg4* using positional cloning techniques (Hauge et al., 2001; Lightfoot and Meksem, 2002). Markers tightly linked to the resistance QTL were used to isolate bacterial artificial chromosomes (BACs) that contained candidate genes. Bacterial artificial chromosomes are large pieces of soybean DNA that have been cloned into bacteria.

In a patent application, Hauge et al. (2001) claim to have cloned candidate genes for *rhg1* and *Rhg4*. Both candidate genes are *Xa21*-like receptor kinases. *Xa21* belongs to class 5 resistance genes (R-genes), and it confers resistance to bacterial blight [*Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al.] in rice (*Oryza sativa* L.) (Zaitsev et al., 2001). *Xa21* is the only known R-gene in this class. Structurally, both candidate genes have an extracellular leucine-rich repeat (LRR) domain, a transmembrane (TM) region, and a serine–threonine

kinase domain (Hauge et al., 2001). The LRR region shows similarity at the amino acid level to the Cf5-like R-gene, *Cf2.1* (LRR-TM class) (Dixon et al., 1996), which confers resistance to leaf mold (*Cladosporium fulvum* Cooke) in tomato (*Lycopersicon esculentum* Mill.). Lightfoot and Meksem (2002) confirmed these results in another patent application. For the *Rhg4 Xa21*-like candidate gene, sequence comparison by both research groups reveals clear differences in the haplotypes between SCN resistant and susceptible germplasm. This is not the case with the *rhg1 Xa21*-like candidate gene, which is monomorphic between PI 88788 and some susceptible southern USA germplasm, such as 'Lee 74', 'Essex' and 'Hutcheson'. Hauge et al. (2001) also found an *HS-1 Pro*-like candidate gene for *rhg1*. *HS-1 Pro* provides resistance to sugar beet cyst nematode (*Heterodera schachtii* Schmidt) isolated from sugar beet (*Beta vulgaris* L.) (Cai et al., 1997). However, the soybean *HS-1 Pro* candidate gene fails to distinguish between resistant and susceptible soybean germplasm. Neither group has reported complementation studies to confirm that they have the correct candidate genes. Thus, it cannot be over-emphasized that these results are preliminary and warrant further validation.

The cloning and analysis of *rhg1* and *Rhg4* should provide invaluable insight into the mechanisms of genetic resistance to SCN and might provide the knowledge needed to engineer novel resistance genes.

### SCN Marker-Assisted Selection

With the availability of genetic markers linked to SCN resistance genes, MAS should increase the efficiency

### SCN Marker-Assisted Selection vs. SCN Greenhouse Bioassay

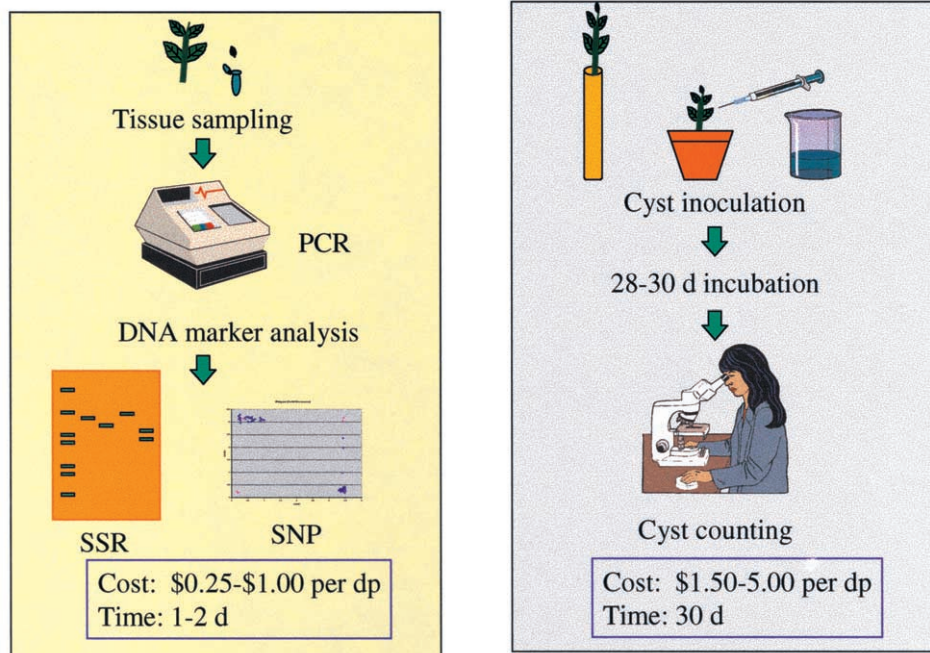


Fig. 3. Comparison between soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) greenhouse bioassay and molecular marker-assisted selection (MAS).



and speed of development of SCN resistant cultivars (Fig. 3). Until now, most breeders have selected resistant lines by inoculating plants in the greenhouse with eggs or cysts of *H. glycines* or by growing soybean progenies in SCN-infested plots. The SCN greenhouse bioassay is labor intensive, costly, and tedious.

The advantage of MAS over conventional SCN screening was echoed by public breeders from throughout the midwestern USA in a survey conducted by Diers and Arelli (1999). They found the majority of breeders believe that the most significant limitation for developing SCN resistant germplasm is the SCN greenhouse assay. With MAS, breeders select lines on the basis of alleles at genetic markers linked to SCN resistance. The lines with a high probability of having resistance genes will be selected, reducing the number of lines that need to be evaluated in the greenhouse. Mudge et al. (1997) showed that with MAS using SSR markers that flank *rhg1*, they were 98% accurate in identifying resistant lines from a cross between 'Evans' and PI 209332. They defined lines as resistant when they had only 30% of the nematode reproduction as their susceptible check. However, MAS for *rhg1* is difficult in some situations because of the lack of polymorphism for many markers linked to this locus when PI 88788 or PI 209332 are compared with some susceptible southern USA germplasm. For example, Satt309, an SSR marker that maps 0.4 cM proximal to the *rhg1* locus, cannot be used in MAS in populations developed from susceptible southern USA cultivars crossed with PI 88788 or PI 209332 because these genotypes all share the identical allele at the Satt309 locus (Cregan et al., 1999b). To address this issue, SSR marker, Sat\_168, which could distinguish PI 88788 and PI 209332 from southern USA susceptible germplasm like Essex, Lee and 'Bragg', was specifically developed from a BAC clone that was identified with Satt309 primers (Cregan et al., 1999b). However, the susceptible soybean lines, 'Hutcheson' and 'Noir-1', share identical alleles with PI 88788 and PI 209332 at Sat\_168 (unpublished observation).

Marker-assisted selection using SSR markers is currently cost-effective compared with greenhouse screening and as marker technologies continue to improve, the cost will likely be further reduced (Fig. 3). Concibido et al. (1996b) estimated the cost of genotyping to be about \$1.50 per data point and requiring less than a week to accomplish from tissue sampling to actual data point generation using RFLP technology. With PCR-based genotyping, the entire process can be done in a day and current cost estimates range from \$0.25 to \$1.00 per data point depending on the efficiency and volume of operation. By contrast, the cost of conventional SCN greenhouse screening ranges from \$1.50 to \$5.00 per plant and still takes 30 d. It is likely that the cost of MAS will further decrease as new genotyping platforms, such as single nucleotide polymorphism (SNP) markers, become more available.

## CONCLUSIONS

Great advances have been made in our understanding of the genetics of SCN resistance at the molecular level

during the past decade. These advances are already benefiting the soybean industry and should lead to future gains. A current benefit of this work is that some breeders are now employing MAS for SCN resistance. In addition, recent cloning of the *rhg1* and *Rhg4* candidate resistance genes (Hauge et al., 2001; Lightfoot and Meksem, 2002) could provide insights on the nature and mechanisms of SCN resistance genes that would be beneficial in engineering novel resistance genes with a broader SCN race spectrum.

Genetic mapping studies have provided information on the locations of major SCN resistance genes in many PIs. Despite the seemingly confusing array of SCN QTL discoveries in over a decade of gene mapping, there is some consistency in the results. In almost all studies, QTL mapping to the *rhg1* region on LG G conferred the greatest resistance. Another important region that was common among many sources was the *Rhg4* region on LG A2. There were four other regions that were significant in more than one genetic background. Minor genes that were reported were suggested to be involved in race specificity but require further validation. There is concern for our seemingly large dependence on one major resistance gene, *rhg1*, which appears to be common among major SCN resistance sources that are currently being used by breeders in North America. It is imperative that we determine whether these sources actually share the same resistance allele.

With the apparent narrow genetic base of SCN resistance in North American soybean germplasm and the intellectual property issues surrounding *rhg1* and *Rhg4*, it is imperative that we evaluate current SCN resistance strategies in breeding programs. One approach is to confirm the efficacy of QTL, other than *rhg1* and *Rhg4*, and better estimate the magnitude of their effects. A second strategy is to search vigorously for novel SCN resistance genes and sources. However, any claim of novelty for newly discovered SCN resistance genes should be verified with appropriate mapping analysis or tests of allelism with *rhg1* and *Rhg4*.

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